

Some Observations on a New Gregarine (*Metamera schubergi* nov. gen., nov. spec.).

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With Plates 15 and 16.

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INTRODUCTION.

WHILE working at Heidelberg in 1906, under Professors Bütschli and Schuberg, the latter kindly called my attention to a new species of gregarine in the gut of *Glossosiphonia complanata* L. (*Clepsine sexoculata*), and suggested its further investigation. The preceding summer, while busied with a recently discovered coccidium occurring in the leech *Herpobdella atomaria* Car. (= *Nephelis vulgaris*), Professor Schuberg turned his attention to *Glossosiphonia complanata*, which occurs in company with *Herpobdella* in the Neckar and occasional ponds in the Heidelberg district. Deeming it probable that two forms so alike in habit and environment might harbour the same parasites, he dissected

several specimens of this leech, and, though the results were in the main negative, he found several animals infected with a species of gregarine. Reference to the literature proved the parasite to be identical with a species briefly mentioned by Bolsius in 1895 (2), and the subject of a more detailed but still fragmentary paper in 1896 (3). Beyond a superficial study carried on incidentally during his work on the *Glossosiphonia* Bolsius seems to have paid no further attention to the parasite, which remained unnoticed until 1900, when Castle (5), in an exhaustive treatise on the N. American *Rhynchobdellidæ* and their parasites, mentions having observed the gregarine seen by Bolsius in about half the specimens of *Clepsine elongata* which he examined. He adds, however, that he only finds the animals in the stomach diverticula, and never in the intestine or crop, as indicated by Bolsius in his diagrams. Castle also mentions encysted protozoa which he found in *C. fusca*, and suggests the possibility of their relationship to the form in *G. complanata*. The cysts he found in the muscle-layers of the body-wall, so that they probably have nothing to do with the gregarine in question.

Lühe (14) quotes the parasite as having been mentioned by Bolsius, and suggests that it probably belongs to the tricytid gregarines.

The gregarine is thus a new and previously undescribed form, for which I propose the name *Metamera schubergi*.¹

In the preparation of the sections and the study of the living animal, during the last few weeks of my stay in Heidelberg, Professor Schuberg assisted me most kindly in every way in his power; and it is due solely to him that I was able to obtain Bolsius' principal pamphlet. My thanks are also due to Geheimrat Prof. Bütschli, whose practical suggestions I found of the greatest value.

¹ The form which appears most closely allied as regards structure of the trophozoite is *Echinomera*. A study of the life-history, however, has revealed points of difference which seem to warrant the creating of a new genus for the form under consideration.

By the kindness of Professor Sedgwick, who allowed me a free hand in the laboratory of the Imperial College of Science, S. Kensington, I was able eventually to complete my study of the sections. And in this connection I must express my indebtedness to Mr. C. C. Dobell, who is at present lecturing at the College. His unrivalled knowledge of protozoan life-history and technique has always been most generously placed at my disposal, and has proved of the greatest value in the preparation of this paper.

MATERIAL AND METHODS.

The leech which serves as host to *Metamera schubergi* is *Glossosiphonia complanata* Linn. A few specimens of *Hemiclepsis marginata* were also found infected. The leeches live under stones in shallow water—running by preference—though I have found them in smaller numbers in still pools. The material was collected at Heidelberg from the shallows left by the summer fall of the Neckar in the neighbourhood of the electric power station, below the new bridge, and also from the opposite bank, along the wall separating the skating rink from the river itself. The leeches are fairly common, and may be found clinging firmly to the under-side of stones at the water's edge, especially in the numerous lumps of red sandstone which litter the shore everywhere.

Recently I examined some specimens of *Glossosiphonia complanata* sent me from the neighbourhood of Cambridge, and found them well-infected.¹ These latter were obtained in January, when the leeches are hard to find owing to the scanty vegetation in the ponds in winter. In all the specimens I examined from this source I only obtained one cyst, and that a very small and early one.

The leeches can be kept for an indefinite period in a good-sized glass jar, provided the water be aerated by passing bubbles of air through it. Food is not necessary, though a

¹ For this I have to thank Mr. Harding, and also for his kindness in assisting me to determine the species.

few small water-snails are much appreciated. Owing to the transparent nature of the integument in *Glossosiphonia*, the parasites are visible in the living leech; and if the latter be forcibly pressed between two slides provided with wax corners, and examined under a low magnification, the gregarines may sometimes be detected in the stomach diverticula and intestine. Unfortunately, however, this method of diagnosis is by no means infallible, as the numerous pigment-cells with their clear nuclei look very like gregarines, and render accurate observation impossible. The gregarines occur in the hindermost stomach diverticula and the intestine, just as indicated by Bolsius in his diagram. The cysts are found in the same regions of the alimentary canal, but are especially numerous in the intestine.

Examination of sections shows that cysts can develop as far as the sporoblast stage in the intestinal canal of the host, though they are often expelled with the faeces at a much earlier stage in development.

In sections just above the anus no cysts were to be seen. This part of the gut was almost occluded by a mass of cephalonts and some sporonts of a peculiarly blunt outline. The leech from which these sections were cut had previously evacuated faeces containing a few very early cysts among a greater number in which sporoblasts could be distinguished. As many as ten cysts have been counted in one section.

To obtain the gregarine, the infected leeches were partially dried on blotting-paper and the under-surface opened by three incisions—two parallel and close to the margins, and one at right angles to the long axis of the animal, at about the junction of the middle and anterior thirds. The flap of tissue was then carefully turned backwards towards the anal sucker, the animal being placed in a watch-glass containing normal saline solution. The gut-contents were thus emptied into the saline, together with connective tissue, which is of no account. By the aid of a hand-lens the gregarines could now be seen sticking to the bottom of the glass, or still fixed to fragments of the host-tissue. These latter are useful in

studying the structure of the epimerite, as this organ, in the course of the teasing out, is very easily torn away, leaving decapitated individuals which may be confused with true sporonts. By gentle coaxing with a pipette the gregarines can be freed from the bottom of the watch-glass and transferred to a slide for further handling.

Preparations in toto were made originally under a cover-slip provided with wax feet, and the various reagents drawn through with blotting-paper. In this way, by fixing the gregarines with alcohol and glacial acetic acid (9 : 1), a large number of animals may be treated under one cover-slip, which is an obvious advantage. More recently I made some preparations by fixing the selected gregarines in a watch-glass with picro-acetic acid (3 : 1) and adding the various fluids by means of a pipette and eventually picking out and mounting the stained gregarines under a low magnification. I consider the former method of treatment the more satisfactory and certainly less laborious. As stains for these preparations I used Grenacher's alcoholic carmine solution and Schuberg's modification of Mayer's acid carmine. This latter solution, being acid in reaction and not neutral, has the power of penetrating the cuticle, and in employing it the preparations must be very rapidly washed through with $\frac{1}{2}$ per cent. solution of HCl to prevent precipitation of the carmine during the further treatment with the alcohols. Leeches destined for sections were fixed either in Gilson's fluid or in the above-mentioned alcohol and acetic mixture. Gilson's fluid should act for two or three hours, and the sublimate constituent be most carefully washed out with iodine-alcohol or a solution of KI in 75 per cent. alcohol. As staining reagents hæmatoxylin (Delafield's) and eosin, safranin, and Heidenhain's iron-hæmatoxylin were employed. Owing to the paucity of material, the laborious expedient of applying both methods in succession on the same preparation had to be employed. It was found that hæmatoxylin and eosin were satisfactory for the cephalonts and sporonts, but gave very incomplete and misleading results with the nuclear changes of the encysted forms, which

were defined much more distinctly with the iron-hæmatoxylin method. All tissues were embedded in paraffin, with chloroform as the intermediary fluid.

Culture of the cysts.—To obtain the ripe spores the cysts were simply placed in the moist chamber, where, in the course of seven or eight days, the spores were developed. The cysts were either placed simply on a slide in a drop of Neckar water or under a cover-slip provided with wax feet. The cysts dehiscid by simple rupture after about seven or eight days. Cysts placed in normal NaCl solution in the moist chamber did not develop successfully.

STRUCTURE OF THE TROPHOZOITE.

The body is divided by septa into epi-, proto-, and dentomerite, and is elongated in form (figs. 1-6). Some individuals have a more thick-set appearance than others, especially in the extreme hinder end of the gut, where the gregarines are often crowded together. The animal measures about 150μ by 45μ . At the posterior end of the dentomerite there are often present indications of further subdivision of the body, and occasionally as many as three complete segments are seen (fig. 4). This segmentation is not confined to gregarines of any peculiar build, being present in both long and short forms, and it varies in the degree of development of the segments. It was present in about a third of the gregarines examined alive in Heidelberg, and is also very distinct in the preparations of these animals made at the time. The Cambridge gregarines also showed segmentation, though it was distinctly less in evidence, both in the living animal and in carmine preparations of it. It appears to vary greatly—from the very faintest indication to quite definite septa. It must be stated in this connection that no segmented gregarines were seen in the sections of the infected leeches, though constantly found in preparations made by teasing out the host-tissues. This compels one to consider the possibility of injury during extraction being the cause of this segmenta-

tion, although the stained preparations do not in the least degree support this suggestion.

The epimerite is a dome-shaped structure. It is provided with short club-like processes, recalling those of *Echinomera*, but often branched, arranged in a dense ring around the line of junction with the protomerite, and also on the roof of the dome (figs. 4 and 5). These latter processes are markedly shorter than those of the ring, and decrease in size as the apex of the epimerite is approached. The processes are perforated at their somewhat clubbed ends by small pores, clearly to be seen in the freshly mounted living gregarine by the aid of a $\frac{1}{12}$ in. oil-immersion lens. Judging from analogy with such forms as *Echinomera* and *Pterocephalus* (Nina), and also from the appearance seen in sections across the point of fixation to the host, there is no doubt that fine pseudopodia are protruded through these pores, which fix the gregarine to the intestinal mucous membrane of the host. The fixing apparatus is by no means easy to identify, as, owing to the unavoidable roughness of the dissection, the gregarines are rudely torn from their moorings, and almost invariably carry away with them a crown-like fringe—derived from the host-cells—which surrounds the epimerite in the zone of the processes, and obscures all details of its structure (fig. 3).

When kept under observation for some time—say an hour or so—in NaCl solution, a curious phenomenon ensues. Just at the line of junction between the protomerite and epimerite a bubble-like vacuole appears, which gradually increases in size, and carries with it the fringe of host tissue with the embedded processes till they sit like a crown on its upper pole, sometimes symmetrically, sometimes displaced to one side. Having reached a diameter about equal to that of the protomerite the vacuole bursts, and the gregarine is suddenly deprived of its epimerite (fig. 2). This vacuole formation has been seen by Léger and Duboscq to occur in *Pyxinia* (14), and in my opinion has a probable bearing on the mooted question regarding the fate of the gregarine epimerite, in the

transition from cephalont to sporont. Frenzel (14) believed the epimerite to be absorbed in a manner similar to the assimilation of a tadpole's tail. He found among numerous cephalonts with large epimerites individuals with but a minute projection from the protomerite, and he regarded this as a scene in the gradual absorption of the epimerite. The sudden disappearance he regarded as pathological, and due to changes in the surrounding medium. My own observations point to the same conclusion. The vacuole formation quoted above is plainly due to plasmoptysis, which can be followed under the microscope from its earliest onset to the bursting of the bubble. Further, when the gregarines were examined in a special solution of egg-albumen, NaCl and camphor, as prepared by Professor Bütschli, the vacuole formation was considerably delayed; a fact explicable on the ground that the solution more nearly resembles the natural environment of the gregarine.

The behaviour of the finger-shaped processes also points to the epimerite being absorbed rather than directly thrown off when the cephalont becomes free. In gregarines which are normally lying free in the gut the processes are never to be seen (figs. 1 and 6). The epimerite is still present, but the processes have been withdrawn during the process of separation from the mucous membrane; just as they are absorbed in *Echinomera* when the cephalont becomes free in the gut (17). This applies to all the free-lying specimens seen in sections, and to a solitary living form which, together with several cysts and some fæces, was pressed out through the anus during examination of a leech between two slides (fig. 1).

In the living sporont (fig. 1) the extreme anterior end of the animal is quite transparent and devoid of granules, a few of which, separate from the main endoplasmic mass of the epimerite, may be seen showing Brownian movement along its anterior border. After some time the whole granular body of the gregarine appears to shrink back somewhat into the cuticular sheath which envelopes it, and this clear area

enlarges proportionally until almost the whole of the conical knob which forms the epimerite is clear of granules. During this process all three divisions of the endoplasm are still quite distinct. By the time this stage has been reached osmosis asserts itself, and the vacuole formation mentioned above commences (fig. 2). In sections, however, the free-lying sporonts all show a curious thickening of the extreme anterior end of the epimerite, which behaves towards stains in the same way as the rest of the cuticle, being, in fact, a thickening of the latter anteriorly (fig. 6). It seems a feasible explanation of this structure to say that it represents the cuticular constituents of the numerous processes of the epimerite, which have been retracted on the animal becoming free. It may here be mentioned that Lühe (14), in his review of the gregarines generally, pronounces in favour of the casting off of the epimerite as the typical way in which the cephalonts become free.

The nucleus lies in the deutomerite. It consists of a nuclear membrane enclosing a clear ground substance, in which lie a large vacuolated karyosome and a number of masses of chromatic substance (fig. 7). The specimens from which figs. 3 and 4 were drawn were very faintly stained owing to excessive washing out, but some other preparations stained with Grenacher's carmine confirm the appearances seen in sections, especially as regards the vacuolated nature of the karyosome. The nuclear area is about 18μ in diameter; the karyosome measures about 8μ , and as a rule contains one very large vacuole and several small ones. The large chromatin masses are scattered irregularly throughout the nucleus, and are of varying shape. The nuclear membrane is well marked, and in common with the karyosome and the chromatin masses stains deeply with both Delafield's hæmatoxylin and Heidenhain's iron-hæmatoxylin. The ground substance takes on a very faint blue tinge with iron-hæmatoxylin. In some of the sections the karyosome has yielded almost completely to the differentiating iron alum, and appears grey by contrast with the black chromatin masses. In

these cases its vacuolated structure is very plain (fig. 7). As a rule, however, the karyosome shows very deeply stained in the adult nucleus. Besides the nucleus there are usually to be seen scattered throughout the body patches of a substance which stains deeply with chromatin stains. These patches have been described by Berndt (1) and others, and are especially numerous in the protomerite. Comes (7) has recently shown that these appearances in *Stenophora* are probably due to metabolic products, and are not nuclear. There are also deeply stained granules in connection with the epimerite processes in sections stained with iron-hæmatoxylin, as described by Schellack in *Echinomera hispida* (17).

CYST-FORMATION AND DEVELOPMENT OF THE SPORES.

The act of association of two animals to form a cyst has not been observed in the living animals. As indicated above, in the sporont the epimerite tends to become less prominent, while a pad of cuticle forms anteriorly. Simultaneously with this shortening of the long axis of the body the protomerite increases in breadth and bulges, particularly around the edges of the apical cuticular pad. From sections it would seem that the two animals come together with their epimerites in contact. A ring of cuticle now arises around the base of the terminal pad in one animal. Into the cup formed by this ring the cuticular pad of the other gregarine is inserted, while external to, and dovetailing with the ring of the cup, a similar ring of cuticle arises in the second animal (fig. 37). In very young cysts in which the nuclei of the two animals are still unaltered the above arrangement of the parts is very clear; but as development proceeds the septum of cuticle dividing the encysted sporonts becomes increasingly irregular. In this region in the earlier cysts there are patches of deeply stained material suggestive of membrane, which are probably the remains of the cuticle of the contiguous epimerites (fig. 13).

Behaviour of the nucleus preparatory to the

formation of the first two daughter-nuclei.—Although the material which I was able to collect was very limited, I was fortunate in obtaining one leech very heavily infected. In the intestine of this animal I found numerous cysts, and also an enormous number of adult gregarines mostly fixed to the gut-wall. A study of these sections has revealed several phases of the first division of the nucleus, though to elaborate all the stages is impossible without further examples, which I hope shortly to procure. In order, therefore, to make the most of this limited material, I employed first hæmatoxylin (DeLafield's) and eosin, and then after decolorisation with acid alcohol, re-stained by Heidenhain's method. This latter method revealed numerous important facts quite indiscernible with the original staining. My thanks are due to Dr. Pembrey, of Guy's Hospital, who very kindly provided me with all the necessary apparatus for staining.

For some time at any rate after a definite cyst-wall has formed, the nuclei of the encysted gregarines remain apparently unaltered. Then the chromatin masses begin to fragment, with the result that chromidia are formed within the limits of the nuclear membrane. Simultaneously, this membrane becomes increasingly thin, and the karyosome throws out masses of substance from its interior, becoming in consequence markedly reduced in size. These masses are more or less spherical and of distinct outline; they stain very deeply, showing black with iron-hæmatoxylin. Their number and size vary greatly (figs. 9–14). At times one large mass is present, almost equal in size to the original karyosome; at others, numbers of small masses are seen. The actual process of extrusion of one of these masses is shown in fig. 36. After their extrusion, the main karyosome-relic shows a blue colour with hæmatoxylin and eosin, as contrasted with the more purple hue shown by the intact karyosome and the chromatin masses of the trophozoite nucleus. The extruded masses on the other hand behave throughout, as regards stains, like the chromatin masses. After the fragmentation of the

chromatin masses and the breaking up of the karyosome have proceeded for some time, a new structure appears in the nucleus. In close proximity to the main karyosome residue, which is seen lying near the periphery of the nucleus, an ill-defined mass appears which takes up nuclear stains very definitely. The earliest appearance of this mass is shown in fig. 9 before the chromidia formation has progressed very far. A slightly later stage is shown in figs. 10 and 11, where the nuclear area presents a homogeneous appearance, without any signs of the chromidial elements being discernible, while the neighbourhood of the main karyosome residue is occupied by a somewhat elongated mass, showing faint longitudinal striation (fig. 11). The relative size of this mass, which I will call the "achromatic mass,"¹ is shown in figs. 9, 10, 11. It will be noticed that the various products of the karyosome are in close connection with it.

At this stage, the absence in my preparations of any structures distinguishable as definite chromosomes or centrosomes is to be emphasised. The achromatic mass stains deeply with iron-haematoxylin, but yields to the differentiating iron-alum before the karyosome and its products become decolorised.

The next stage in the division represented is shown in figs. 12 and 13. The achromatic mass has increased in bulk and definition, and has become more drawn out. The striation is very marked, and for the first time in the course of the division the true chromosome element appears. At each pole of the achromatic mass, which is now distinguishable as a true spindle, there is a small black mass of chromatin; while converging towards this mass, like the ribs of a basket, are seen deeply stained streaks of granules of chromatin, arranged upon the spindle-fibres and obviously en route for the respective poles of the figure. It may here, again, be seen that the spindle stains very deeply with chromatin stains, and

¹ I call this structure the "achromatic mass" because of its function—as seen in its later development—and not on account of its staining properties.

it is only on very thorough differentiation that the chromosomes are rendered visible. The spindle fibres appear to merge with the terminal chromatin mass. Distal to this there is no true astral arrangement visible.

Each terminal chromatic aggregation now gives place to a definite vesicular structure, situated at the poles of the spindle and forming the centre of a definite astral radiation (figs. 14 and 15). Simultaneously with the appearance of the vesicle, the chromatin streaks and granules disappear from the spindle, so that the more definite the terminal vesicle, the fewer the chromosomes on the spindle. Fig. 12 shows a ring-like arrangement of the terminal chromatin aggregation at one pole of the spindle (*a*), while fig. 15 shows a true polar vesicle containing definite granules of chromatin, in one instance arranged indiscriminately around the circumference, in the other accumulated at one point upon it. These vesicles are the points upon which the very definite spindle-fibres converge, and measure from $1\frac{1}{2}$ – $2\frac{1}{4}$ μ across. In figs. 14 and 15 it will be noticed, firstly, that—apart from the granules within the vesicles and the karyosome products—there are practically no other discrete chromatin elements to be seen; secondly, that some of the spindle-fibres plainly run down into the midst of the nuclear area and the karyosome remnants, where these latter are not already lying on the spindle. In fig. 15 will be seen, lying close to the large irregular karyosome residue, a collection of deeply stained granules, which are connected with the karyosome and with each other by deeply stained strands. They have probably been recently thrown out from the karyosome, which is much distorted from its original spherical shape.

The latest stage of the first division represented among my slides was unfortunately injured before anything more than a rough drawing had been made of its structure (fig. 16). It represented the spindle very much drawn out, just before the final separation of the two daughter-nuclei. There was at each pole a well-marked vesicle, containing numerous granules of chromatin, and distal to this vesicle was a mass of achro-

matic substance, showing within it a granule of deeply stained substance. The figure was very suggestive of the state of affairs seen in fig. 18 *a* and *b*, with, however, a single polar granule. The sparsity of material unfortunately renders a complete account of the first division-phenomena out of the question. From a careful study of the slides at my disposal I suggest the following as the more striking points, the significance of which I shall revert to later on (see p. 278). Firstly, the depth to which the spindle proper stains with both Delafield's and Heidenhain's hæmatoxylin: secondly, the proximity of the karyosome to the origin of the achromatic mass, and, later on, the very definite spindle-fibres running down in among the karyosome remnants and the site of the old nucleus: thirdly, the absence of regular chromosomes such as can at any stage be outlined or counted with anything approaching certainty: fourthly, the vesicles at the poles of the later spindles, which form the centres of definite astral figures. The nature of these vesicles it is difficult to decide. Are they centrosomes or incipient daughter-nuclei? As will be seen later, the daughter-nuclei are strikingly vesicular; and the fact that, if these vesicles are considered as centrosomes pure and simple, there are no other defined chromatic elements in the spindle figure, seems to indicate their being early stages of the daughter-nuclei. This being the case, the centrosome must be sought either in one of the granules on the circumference of the vesicle, or distal to the latter. On this point, though tempted to an explanation, I dare not base a theory upon a drawing so diagrammatic as fig. 16.

Proceeding to the further division of the daughter-nuclei, all uncertainty about the centrosome vanishes. In the earliest stages, where eight or nine nuclei are present in each cyst (fig. 17 *a*, *b*, and *c*), the astral radiations are very marked, and the centrosome consists of a deeply stained mass at the periphery of the nuclear vesicle, from which emanate the striæ. These, where they spring from the centrosome, are extremely obvious. In fig. 19 *c* and *d*, stained with hæma-

toxylin and eosin, the centrosome is differentiated into a faintly stained peripheral portion—the centrosphere—in the centre of which is a black centriole; this also shows in fig. 20 stained in the same manner.

In studying the various generations of daughter-nuclei several interesting points demand attention. They present an infinite variety as regards the arrangement of their chromatin. Except when actually drawn out into a spindle they are invariably vesicular in structure; and, in the great majority of cases, in the earlier stages at any rate, they contain a distinct karyosome. This is of interest in that in *Echinomera hispida*, described by Schellack (17), where the karyosome invariably appears in the daughter-nuclei, its origin is referred to the unpaired chromosome of this form, which chromosome thus has a function allotted to it. In *Stylorhynchus*, which also shows this phenomenon, there is, however, no such unpaired chromosome (11). The fate of these daughter-karyosomes in *Metamera schubergi* is not certain. The corresponding spindle figures do not show any traces of karyosome fragments in their neighbourhood. On the other hand, in such stages as shown in figs. 19 *c* and *d*, where the nucleus is on the point of elongating into a spindle, the karyosome seems to be extruding part of its substance. If this is so, the process is one of immediate and complete solution, and not exactly parallel with the behaviour of the adult karyosome. It must be clearly understood that, as the figures show, a karyosome cannot be always with certainty identified in these daughter-nuclei. There are always present masses of chromatic substance of varying sizes, and their arrangement is at times such as to make the distinction impossible. In the daughter spindle-figures, as with the first division, there is again no definite chromosome formation. The chromatic elements are sometimes discernible as streaks and granules near the poles of the spindle; sometimes the deep black appearance of the spindle-fibres, alone present, suggests that these latter may be conveying chromatin in very minute particles. A constant feature of these young

spindles is a black mass of deeply staining matter at the extreme poles. In some early spindles shown in fig. 18 *a*, the earliest actual daughter spindle-stage to hand, this polar mass is seen as two adjacent granules or centrioles lying in a definite centrosphere showing radiations. In fig. 18 *b* these two granules are connected by a deeply staining link. This I interpret as the early division of the centrosome, occurring almost before the daughter-nuclei, which in the figs. 18 *a* and *b* are distinguishable as faint vesicles, are free from their parent spindle. In this connection it is of interest to note that the daughter-nuclei always appear provided with two centrosomes. I have not been able to discover any with a solitary centrosome. This is in keeping with the above suggestion as to the early division of the centrosome in the history of each daughter-nucleus. As the daughter-nuclei become smaller their division-figures become less complicated, while the chromatin becomes arranged as a single mass rather than as separate particles. Some of the smallest spindles still show occasionally distinct chromatin elements near their poles, but the majority do not. There appear to be no definite astral rays distal to the terminal mass of chromatic substance (figs. 21 *d*, 23, and 24 *b*). Finally all traces of spindle-formation disappear, and the nuclei are reduced to mere masses of chromatin about 1 to 1.5 μ in size. These are arranged on the periphery of masses of protoplasm, after the fashion of a typical so-called Perlenstadium, and the protoplasm soon becomes mammillated round each nucleus with the formation of gametes (fig. 25).

That part of the protoplasm which does not take part in the formation of the gametes—the Restkörper—contains a few nuclei which have not kept pace with the general division (fig. 25 *b*). These laggard nuclei are present here and there in all sections of the later daughter-divisions, and are noticeable in that they are larger than their more numerous companions. Similar nuclei have been noticed by Léger and Duboscq in *Hoplorhynchus* (13). Scattered throughout the later cysts are also seen a number of round clear bodies

(fig. 25*a*) stained very faintly with iron-hæmatoxylin. They are most obvious in cysts containing gametes or sporoblasts, and have not been seen in the earlier cysts, at any rate in the same form. Their size varies considerably, and they appear to be products of the original karyosome which have lost most of their staining properties, and which have become more obvious owing to the splitting up of the protoplasm entailed in gamete formation. The majority are rather too large to be referred to the daughter-karyosomes. The main residue of the original karyosome is often to be found, deeply stained, in these later cysts.

The gametes are very like those described for *Lankesteria ascidiæ* by Siedlecki (18), and show no signs of sexual differentiation (fig. 26). Considering the fact that there is at no time in the history of the encysted animals any difference in structure, and that the nuclear changes are practically coincident, this isogamous type of gamete is what one would expect. Conjugation has not been observed in the living animal, owing to my studies being interrupted by my departure from Heidelberg. Fig. 27 shows, however, what is practically certain to be a zygote. The gametes measure about 3μ , and are roughly circular in outline. Their nuclei consist of small masses of chromatin with no definite vesicular structure. The zygote measured over 4.5μ , and contained two distinct nuclei. Several cysts were found containing sporoblasts, (figs. 28 to 33). These are ovoid bodies measuring 6μ by 4μ , and containing large vesicular nuclei. These sporoblasts gradually acquire a spore coat, and grow in size somewhat during the process (fig. 33), so that in a cyst of sporoblasts one or two may be detected with the outline of a formed spore (fig. 34). The fully formed spore is shown in fig. 35. The nuclear changes resulting in the formation of the sporozoites have not been made out, nor did I obtain a view of a free sporozoite. It was easily seen, however, in optical sections of the living spores that eight sporozoites were arranged peripherally around a granular mass of residual protoplasm. The spores measure 9μ by 7μ , and are navicelliform, provided at each end with a little peg-like projection (fig. 35).

DISCUSSION OF SOME SPECIAL POINTS IN THE LIFE-CYCLE.

In the description of the trophozoite mention has been made of the traces of further segmentation shown occasionally at the posterior end of the deutomerite in *Metamera schubergi*. The presence of segmentation in some gregarines, apart from the three fundamental divisions of the body, is a well-established fact, Léger (12) having described a form, *Tæniocystis*, where this phenomenon is so well marked as to make the animal resemble a small cestode. *Porospora* (13) also shows a segmentation, which, however, appears to be somewhat different in nature, as the animal is said to be capable of obliterating its segments merely by stretching itself out during movement.

In *Metamera schubergi* the segmentation is always confined to the posterior end of the deutomerite, and is not constantly present. In their full development these posterior septa appear in every way as definite as those of the anterior part of the gregarine; but in some animals, on the contrary, it requires the most careful focussing to demonstrate their existence. I am unable to explain the significance of these septa; whether they mark a certain period in the life-cycle or whether they are due to some form of plasmolysis I cannot say. They are, however, sufficiently often present to form a striking feature of this gregarine.

As regards the explanation of the phenomena shown in the division of the nucleus, it is difficult to discover anything of the nature of a precedent in the current description of this stage. The vacuoles described by Cuénot (6), Prowazek (16), and others, in close proximity to the sporont nucleus, or by Siedlecki (18) within the latter, have not been seen in *Metamera schubergi*. From the proximity of the commencing achromatic mass to the actively disintegrating karyosome, I suggest that this latter body supplies material—more or less, it is impossible to say—which will assist in the formation of the two daughter-nuclei. Another point, to

which attention has been frequently called, is the intense staining capacity shown by the achromatic mass, both at its first appearance and later in the fully formed spindles. This applies equally to Delafield's hæmatoxylin and to Heidenhain's method, which latter is known to stain plastin-substance darkly. Now the chromosome material, when first detected, is seen as streaks lying on the spindle-fibres near the poles; or, when the fibres are seen in optical section, as a line of contiguous granules (figs. 12 and 13). No preparation showing an equatorial arrangement of the chromosomes was obtained, although, of course, this does not prove the non-existence of such a stage. Fig. 12 shows some of the chromatin streaks directly continuous with the well-marked terminal mass; and it is thus possible that this mass represents a collection of chromatin which has been delivered by the spindle-fibres. I suggest, therefore, that throughout the division the spindle-fibres are carrying chromatin in a form unrecognisable as discrete particles, until it undergoes condensation towards the poles of the figure. With the appearance of the vesicles the chromatin elements disappear from the spindle, leaving only the few scattered granules of figs. 12 and 15. These vesicles would thus appear to have been formed from the chromosomes of the earlier stages, and supposing them to be indeed daughter-nuclei, it is conceivable that they go on growing at the expense of chromatic substance still uncondensed in the spindle-fibres, until finally they become free as the first pair of daughter-nuclei. This theory would account for the staining properties of the spindle; and the absence, at the earliest stage of the division, of definite chromosomes.

As regards the origin of the chromatin of the daughter-nuclei, there is nothing upon which to dogmatise. We have the fragmentation of the original chromatin masses, which proceeds until the resultant particles are indistinguishable, and we have the breaking-up of the karyosome, both of which might supply a source for the chromatin. That this chromatin is being in some way drawn up on to the spindle

from the débris of the old nucleus is obvious from figs. 14 and 15.

Siedlecki, in his work on the karyosome of *Caryotropha* (19), reviewing the rôle played by this body in *Coccidia*, points out that while in some types the karyosome plays a purely vegetative part, in others it has definite responsibilities regarding the reproductive functions. The latter appears to be the case in *Metamera schubergi*. If, as I believe to be the case, the daughter-nuclei reform their karyosomes, may not these daughter-nuclei—which are, after the upheaval of the trophozoite nucleus during its first division, presumably sexual in nature—throw some light on the functions of the karyosome? If the latter be purely vegetative in function, why should it recur in the daughter-nuclei, which, with their two centrosomes, are plainly not in a vegetative condition?

In the face of the facts it is certainly a reasonable suggestion that the original karyosome consists of two elements at least. The one of these is thrown out at the first division of the nucleus, and is of no further use in the formation of the daughter-nuclei; the other is of vital importance in the propagation of the species, as realised in the sexual gametes. In the daughter-karyosomes only one of these components persists—i. e. that part essential to nuclear division; the other part—for which, in the active reproductive processes now proceeding no need remains—is not represented. Thus, in the daughter-spindles no karyosome remnants are seen. This is hardly the place for a discussion on the binuclearity hypotheses, so ably dealt with by Dobell (8), but the above-mentioned differentiation of the karyosome constituents is sufficiently suggestive. On the one hand, the vegetative and reproductive elements of Goldschmidt's theory may be seen in the original karyosome residue and the so-to-speak more intense daughter-karyosome respectively. On the other hand, one is equally justified in assuming that the karyosome residue merely represents elements whose life is over and whose functions are exhausted, while the perpetuated remainder persists in the daughter-karyosomes, which are

thus thoroughly equipped for their part in the ceremony of division.

It will be noticed that, except in fig. 15, where the vesicles attain their maximum development, there is no true striation shown distal to the polar aggregation; in other words, although the spindle-fibres are throughout very distinct, the centrosome element is not. This, again, suggests a bearing on the origin of the centrosome. On the one hand, as Dobell (8) points out, we have a binucleate condition held as the starting-point in the development of the centrosome; on the other there are observers, such as R. Hertwig, who believe the centrosome to be a specialisation of the central spindle, so that the spindle in the Protozoa is equivalent to centrosome + spindle of the Metazoa. Without wishing to claim originality for the suggestion, I may say that the first division figures of *Metamera schubergi* have all along pointed forcibly to a most interesting lack of differentiation and specialisation between the various constituents. The chromatin is not marked off in the form of distinct chromosomes, nor are the centrosomes—assuming my interpretation of the figures to be correct—distinguishable as such. The three elements, chromatin, spindle, and centrosome, act in concert in the formation of the first two daughter-nuclei, and it is difficult to say where one begins and the other ends. I suggest, therefore, that the evidence afforded by *Metamera schubergi* tends to support Siedlecki's view, expressed in connection with his work on *Caryotropha* (8), that "we have in a protozoan cell . . . but a single and simple nuclear apparatus before us," and not a binuclear arrangement.

In conclusion, with reference to the apparent isogamy shown by this gregarine, it will be noticed that we have another apparent exception to what Léger (13) deems the general rule in gregarines, i. e. anisogamy. In this connection the recent work of Brasil (4) and Hoffmann (10) on *Monocystis*, which had previously been considered isogamous, is interesting. The work of the latter emphasises the futility of drawing conclusions from stained preparations.

He showed that a very definite anisogamy was visible in the living cysts, which, however, became much less marked in the process of fixing and staining. This may be so in *Metamera schubergi*, but, considering isogamy as the more primitive condition, it is possible that this gregarine, whose first spindle suggests a phase in the evolution of karyokinesis, may also exhibit true isogamy.

I hope in the spring to renew my acquaintance with this species, and to be able to complete its life-history.

DIAGNOSIS OF *METAMERA SCHUBERGI* N.G., N.SP.

A cephaline gregarine belonging to the family Dactylophoridae (Léger).¹ Trophozoite ca. 150 μ by 45 μ . Epimerite subconical, with apex excentrically placed, and surrounded by numerous branched, digitiform appendages. The dentomerite sometimes (not always) shows a secondary septation into one to three segments in the region posterior to the nucleus. Conjugation isogamous, no sexual differentiation being observable at any stage in the life-cycle. Cyst dehiscing by simple rupture. Spores navicelliform, containing eight sporozoites, and measuring 9 μ by 7 μ .

Hosts: *Glossosiphonia complanata* (Heidelberg and Cambridge) and *Hemiclepsys marginata* (Heidelberg).

GUY'S HOSPITAL,

LONDON, S.E.,

February, 1910.

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EXPLANATION OF PLATES 15 AND 16,

Illustrating Mr. H. Lyndhurst Duke's paper on "Some Observations on a New Gregarine (*Metamera schubergi* nov. gen., nov. spec.)."

PLATE 15.

[Figs. 1 and 2 were drawn from living animal, figs. 3 and 4 from preparations fixed with alcohol and acetic acid and stained with Schuberg's modification of Mayer's acid carmine. Figs. 5, 6, and 16 are diagrammatic. Figs. 7-18 were fixed with Gilson's fluid and stained with Heidenhain's iron-haematoxylin. All these figures were drawn with Zeiss oc. 6, obj. 2 mm. apochromatic. Figs. 9, 10, 11, and 17, 18 are to scale at magnification of 2000. Figs. 12, 13, 14 are drawn on a slightly smaller scale.]

Fig. 1.—Living sporont expressed through anus of leech.

Fig. 2.—Same sporont as Fig. 1, showing bubble-formation.

Fig. 3.—Cephalont with epimerite embedded in fragment of host-tissue.

Fig. 4.—Showing optical section of epimerite.

Fig. 5.—Diagram of structure of epimerite, etc.

Fig. 6.—Diagram of sporont with cuticular pad on epimerite.

Fig. 7.—Nucleus of trophozoite.

Fig. 8.—Nucleus showing fragmentation of chromatin masses and extrusion process of karyosome.

Fig. 9.—Sporont nucleus showing earliest appearance of the "achromatic mass," with fragmentation of the karyosome.

Figs. 10 and 11.—Successive sections of another nucleus showing slightly later stage than fig. 9.

These three figures (9, 10 and 11) are drawn from same cyst.

Fig. 12.—First division of sporont nucleus showing at (*a*) the ring arrangement beginning at the pole; also the streaks of chromatin and the spindle-fibres in optical section. The two poles are respectively at the extreme upper and lower focus. One of the chromatin streaks is seen running into the polar aggregation.

Fig. 13.—An early cyst, containing two associated individuals, with remains of epimerites seen at the centre. Nuclei at stage of first division. In upper animal the polar aggregation and the chromatin streaks are very marked. (Combined from two successive sections.)

Fig. 14.—First division of the sporont nucleus at a somewhat later stage than figs. 12 and 13. Shows polar vesicles more distinct. Also the distinct fibres running down into neighbourhood of original nucleus and karyosome.

Fig. 15.—First division of sporont nucleus at a later stage than fig. 14. Vesicles fully formed and fibres running down towards karyosome. The vesicles here shown were 6μ apart, lying respectively at top and bottom focus.

Fig. 16.—Diagram of first spindle just before final separation of first two daughter-nuclei.

Fig. 17, *a*, *b* and *c*.—Earliest stage of daughter-nuclei, eight or nine in cyst.

a. Shows centrosomes connected by a thick band.

b. Shows chromatin bunched as an early spindle figure.

c. Shows karyosome.

All from same cyst.

Fig. 18.—Somewhat later daughter-nuclei at end of division.

a. Shows two centrioles at each pole; also one daughter-vesicle. (The section has not passed through the left vesicle.)

b. Shows division of the centriole with poorly developed daughter-vesicle. (The vesicle at the right end of the figure lies outside the plane of this section, and is therefore not seen.)

c. Shows a separated daughter-vesicle.

All from same cyst.

PLATE 16.

[Figs. 21-34 and 36 were fixed with Gilson's fluid and stained with Heidenhain's iron-haematoxylin. Figs. 19 and 20 were stained with Delafield's haematoxylin and eosin. Figs. 19-34 were drawn at magnification of 2000. Fig. 35 is not to scale, being relatively too large.]

Fig. 19 (*a-e*).—From same cyst. Somewhat later daughter-nuclei. All show karyosomes. *c* and *d* show early stage of spindles, and the karyosomes in a state of activity.

Fig. 20.—Showing differentiation of centrosome into centriole, and centrosphere in a daughter-nucleus of same stage as fig. 19.

Fig. 21.—Similar daughter-nuclei showing karyosomes: also corresponding spindle.

Figs. 22 and 23.—Later stages of daughter-nuclei, mostly showing karyosomes; also corresponding spindles.

Fig. 24.—Smaller daughter-nuclei and spindles.

Fig. 25.—Shows the Perlenstadium, with a single free gamete. Notice the clear karyosome remnants (*a*), and the residual nuclei (*b*).

Fig. 26.—Gametes.

Fig. 27.—A zygote with two unfused nuclei.

Figs. 28-32.—Sporoblasts. Figs. 29 and 31 show these in transverse section.

Fig. 33.—Shows a sporoblast assuming shape of spore.

Fig. 34.—Shows a spore coat in process of developing.

Fig. 35.—Fully formed spore, with sporozoites in optical section.

Fig. 36.—Shows the karyosome in the act of extruding some of its substance.

Fig. 37.—Diagram to show method of apposition of associating sporonts in a cyst.